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THIOREDOXIN *h* CONSTRUCTS FOR TRANSFORMATION

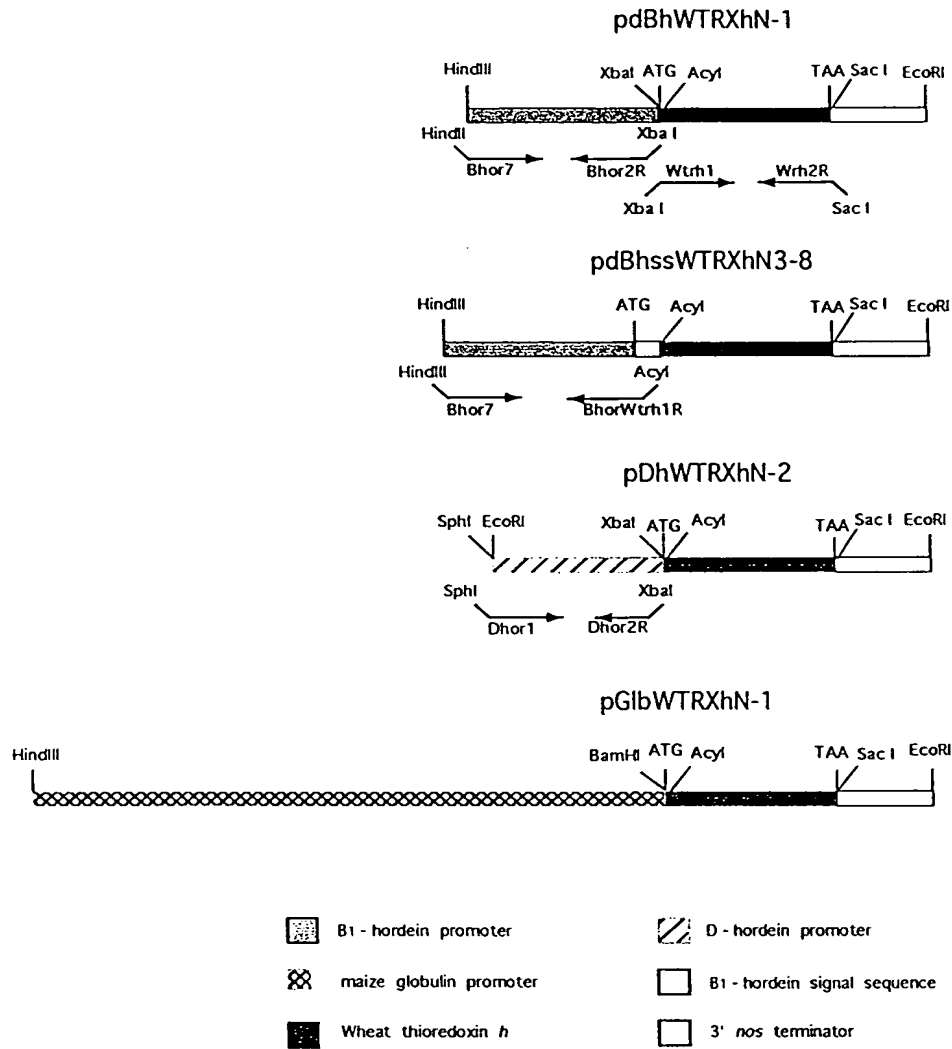
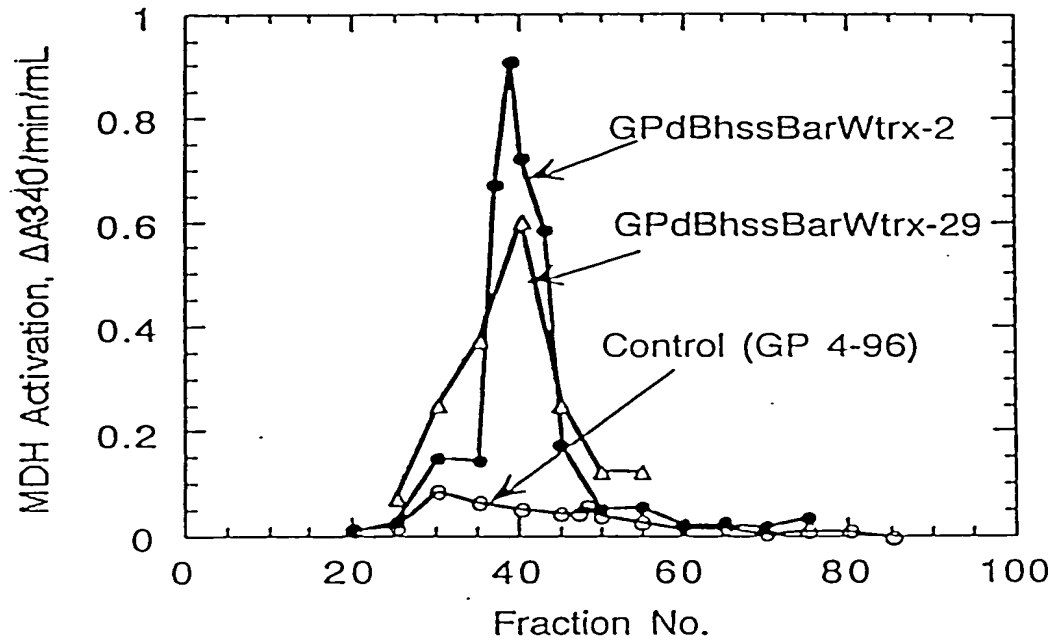


Figure 1

Sephadex G-50 Activity Profile of Thioredoxin from Barley Grains (+MDH)



Sephadex G-50 Elution Profile of Proteins from Three Barley Grains

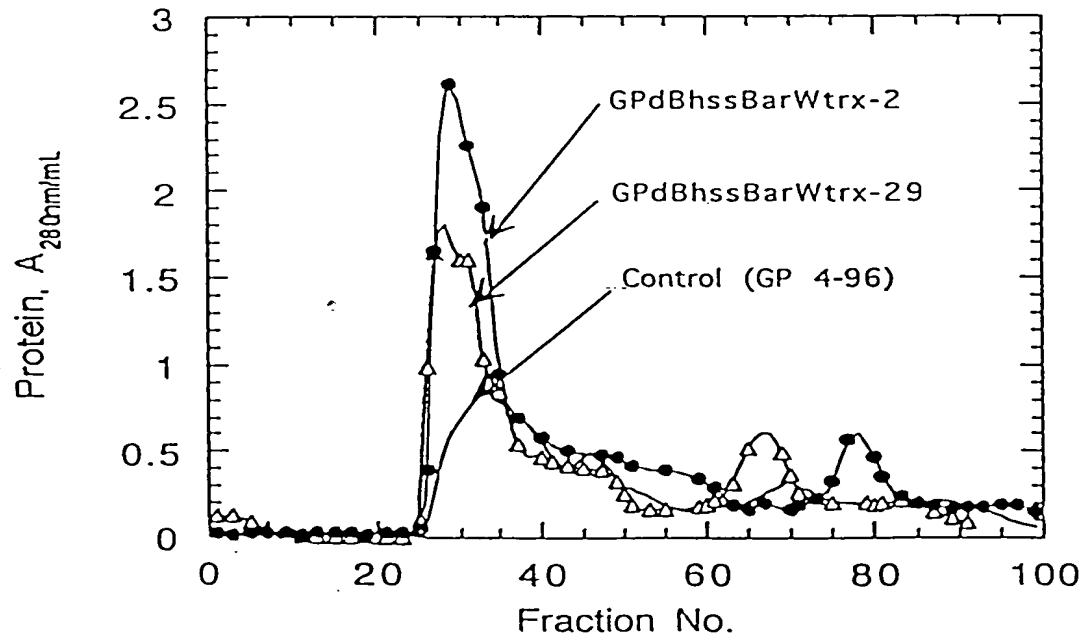


Figure 2

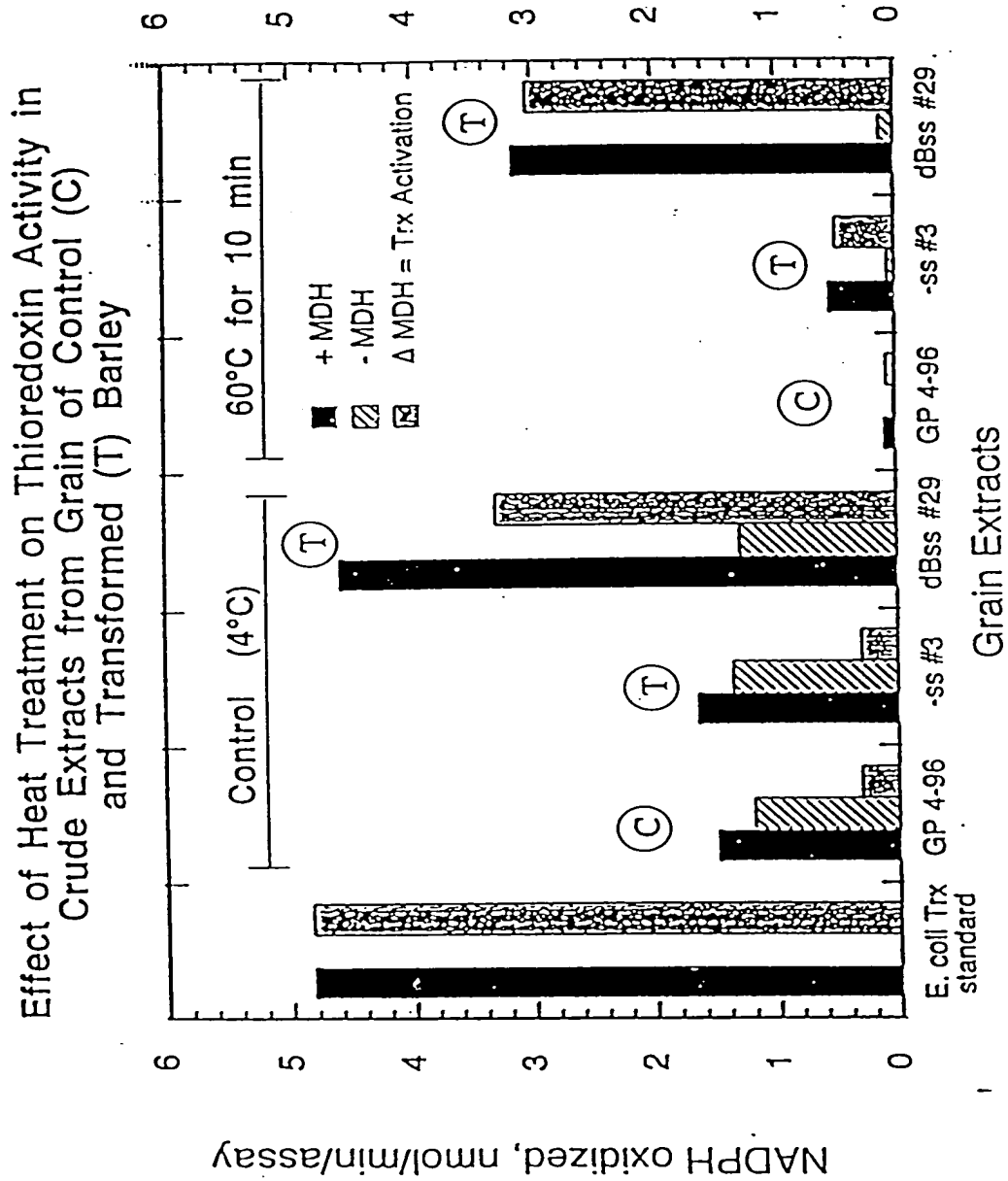


Figure 3

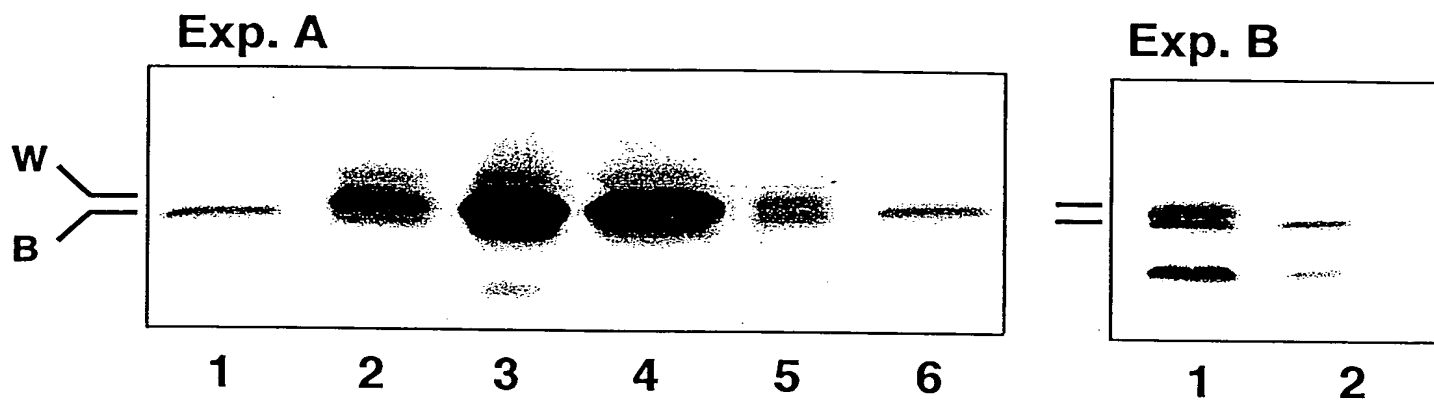


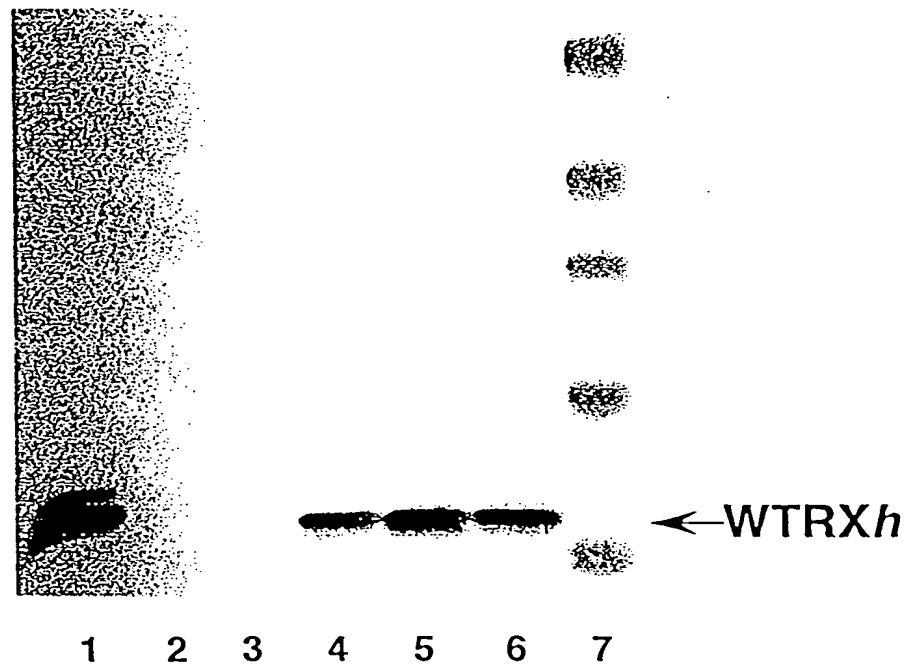
FIGURE 4

Western Blot Analysis of Barley Grain Transformed with Wheat Thioredoxin

SDS-PAGE: cv. Golden Promise

Title: VALUE ADDED TRAITS IN GRAIN AND
SEE TRANSFORMED WITH THIOREDOXIN
Inventor: Myeong-Je CHO et al.
Application No.: Not Yet Assigned

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1. Wheat germ thioredoxin
2. Control (GP 4-96), nontransformed
3. Control, null segregant (GPdBssBarWtrx-29-11-10)
4. Transformed, heterozygous line (GPdBssBarWtrx-29)
5. Transformed, homozygous line 1 (GPdBssBarWtrx-29-3)
6. Transformed, homozygous line 2 (GPdBssBarWtrx-29-3-2)
7. Prestained standards

FIGURE 5

AAGCTTTAACAACCCACACATTGATTGCAACTTAGTCCTACACAAGTTTTCCATT
 CTIGTTTCAGGCTAACAACCTATACAAGGTTCCAAAATCATGCAAAAGTGATGC
 TAGGTTGATAATGTGTGACATGTAAAGTGAATAAGGTGAGTCATGCATACCAAA
 CCTCGGGATTTCTATACTTTGTGTATGATCATATGCACA'CTA'AAAGGCAACTTT
 GATTATCAATTGAAAAGTACCGCTTGTAGCTTGTGCAACCTAACACAATGTCCA
 AAAATCCATTTGCAAAAGCATCCAAACACAATTGTTAAAGCTGTTCAAACAAAC
 AAAGAAGAGATGAAGCCTGGCTACTATAAATAGGCAGGTAGTATAGAGATCTA
 CACAAGCACAAGCATCAAAACCAAGAAACACTAGTTAACACCAATCCACTATGA
AGACCTTCCTCATCTTTGCACTCCTCGCCATTGCGGCAACAAGTACGATTGCA

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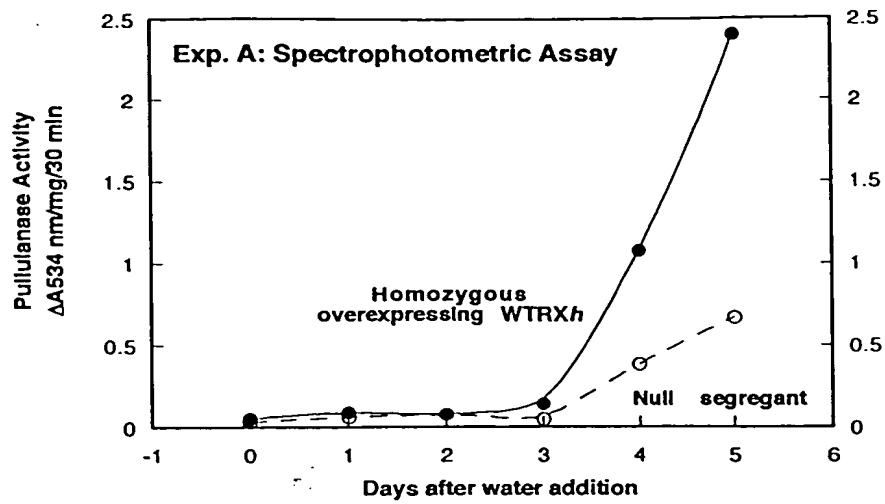
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FIGURE 6

CTTCGAGTGCCCCGCCGATTTGCCAGCAATGGCTAACAGACACATATTCTGCC
 AAAACCCCAGAACATAATCACTTCTCGTAGATGAAGAGAACAGACCAAGAT
 ACAAACGTCCACGCTTCAGCAAACAGTACCCCAGAACTAGGATTAAGCCGAT
 TACGCGGCTTTAGCAGACCGTCCAAAAAAACTGTTTTGCAAAGCTCCAATTCC
 TCCTTGCTTATCCAATTTCTTTTGTGTTGGCAA'ACTGCACTTGTCCAACCGATT
 TTGTTCTTCCCGTGTTTCTTCTTAGGCTAACTAACACAGCCGTGCACATAGCC
 ATGGTCCGGAATCTTCACCTCGTCCCTATAAAAGCCCAGCCAATCTCCACAAT
 CTCATCATCACCGAGAACACCGAGAACCACAAA'ACTAGAGATCAATTCATTG
 ACAGTCCACCGAGATGGCTAAGCGGCTGGT'CCTCTTTGTGGCGGTAATCGTC
GCCCTCGTGGCTCTCACACCGCT

FIGURE 7

FIGURE 8



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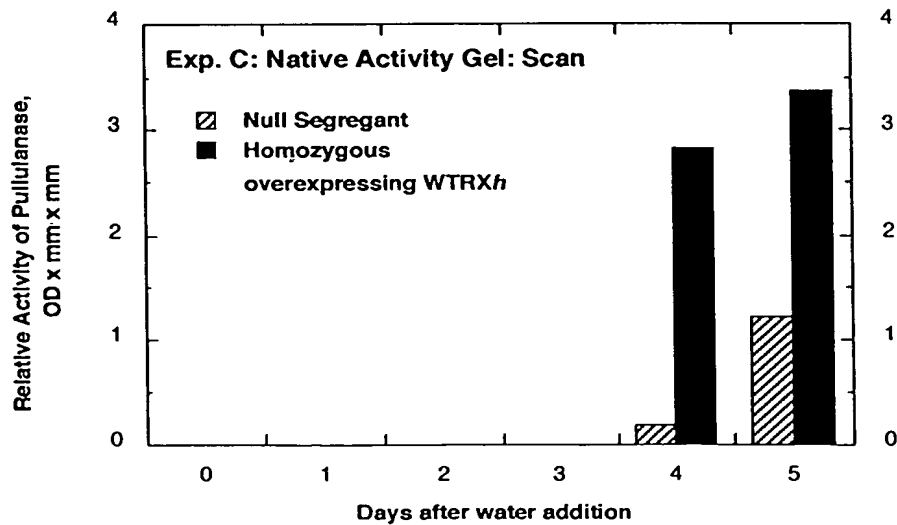
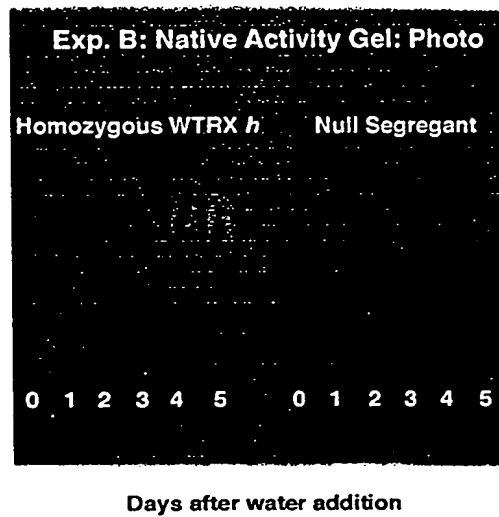


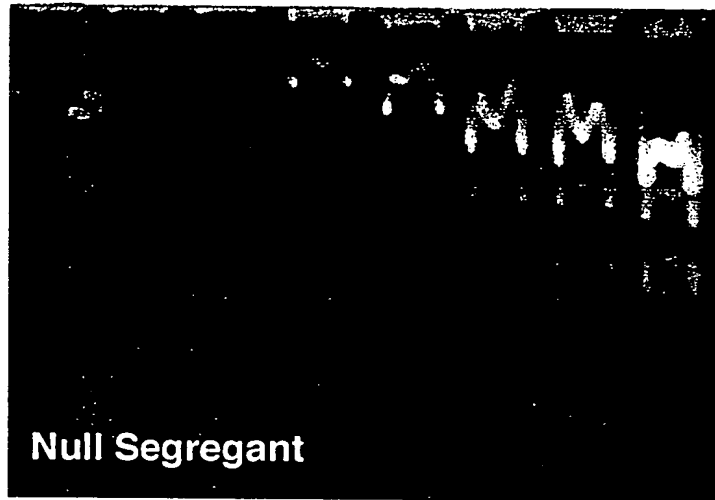
FIGURE 9

**Abundance of
 α -amylases**

0 1 2 3 4 5 6 7

A

**Total Amylase
Activity**



B

0 1 2 3 4 5 6 7

C



D

Figure 10

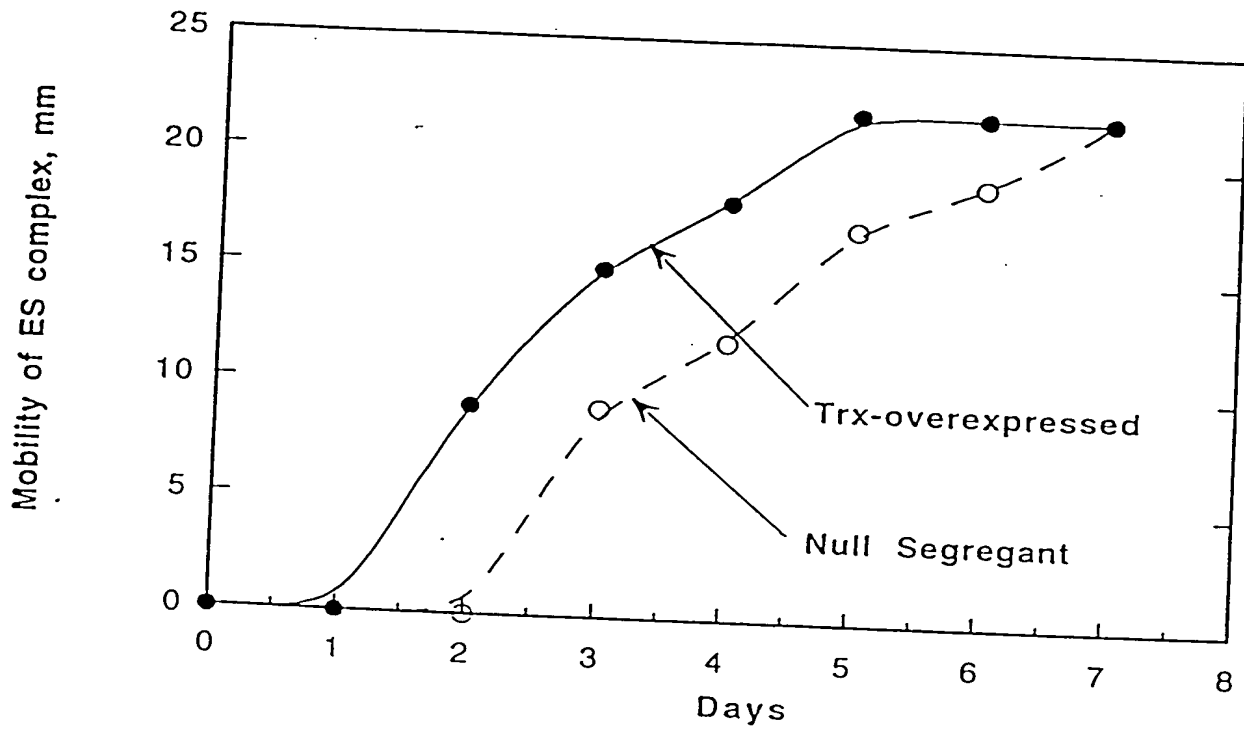


FIGURE 11A

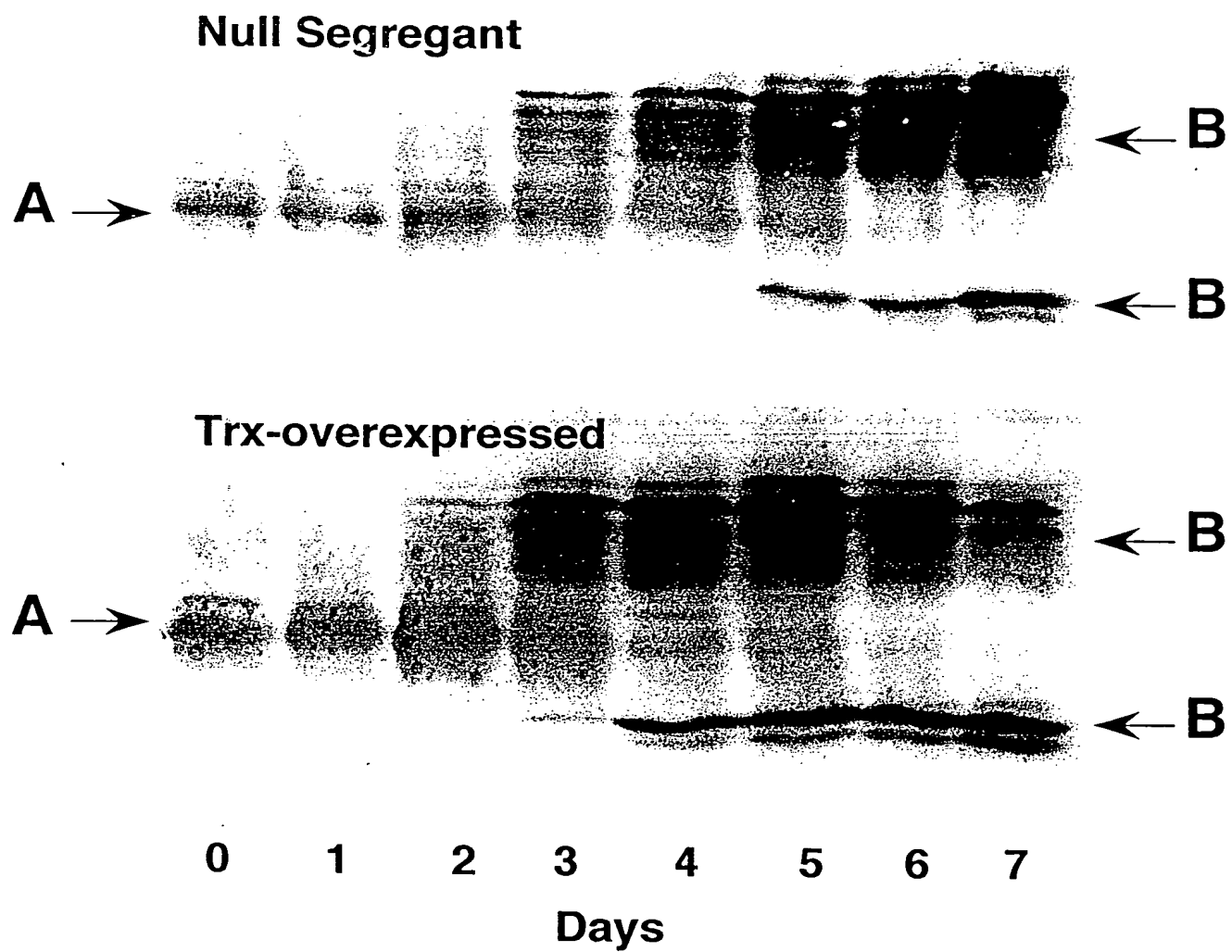


FIGURE 11B

DNA CONSTRUCTS FOR TRANSFORMATION

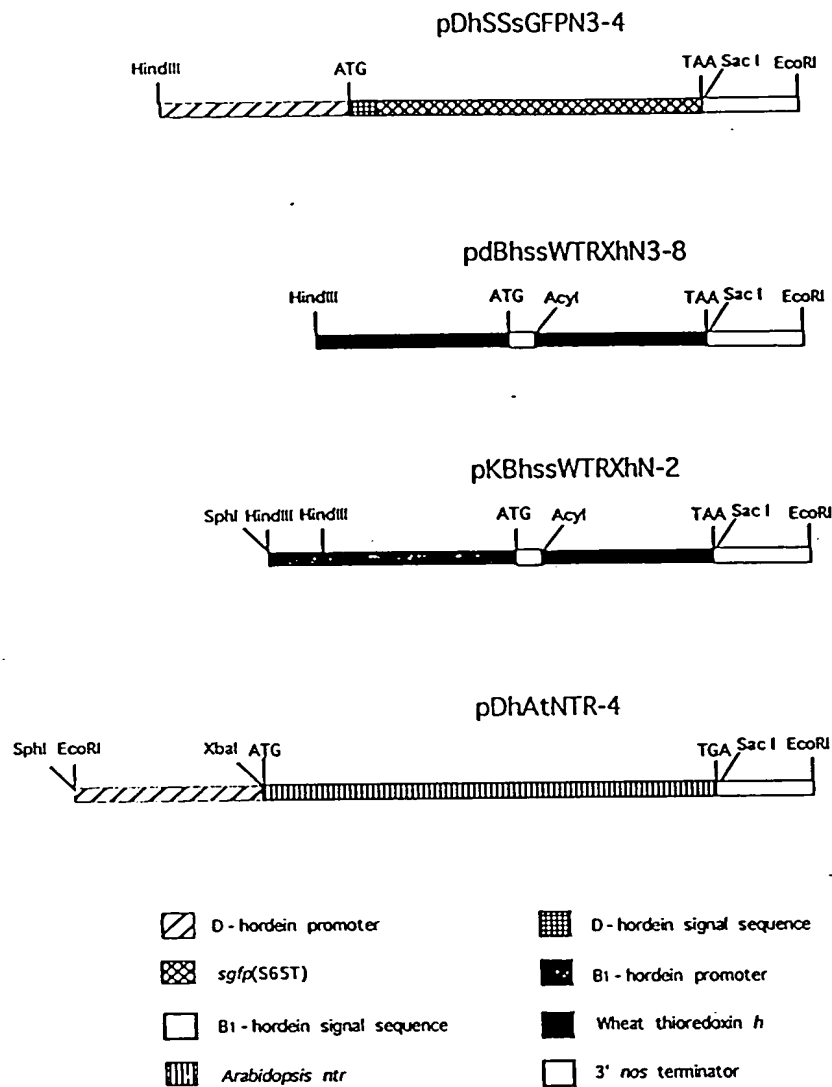


Figure 12

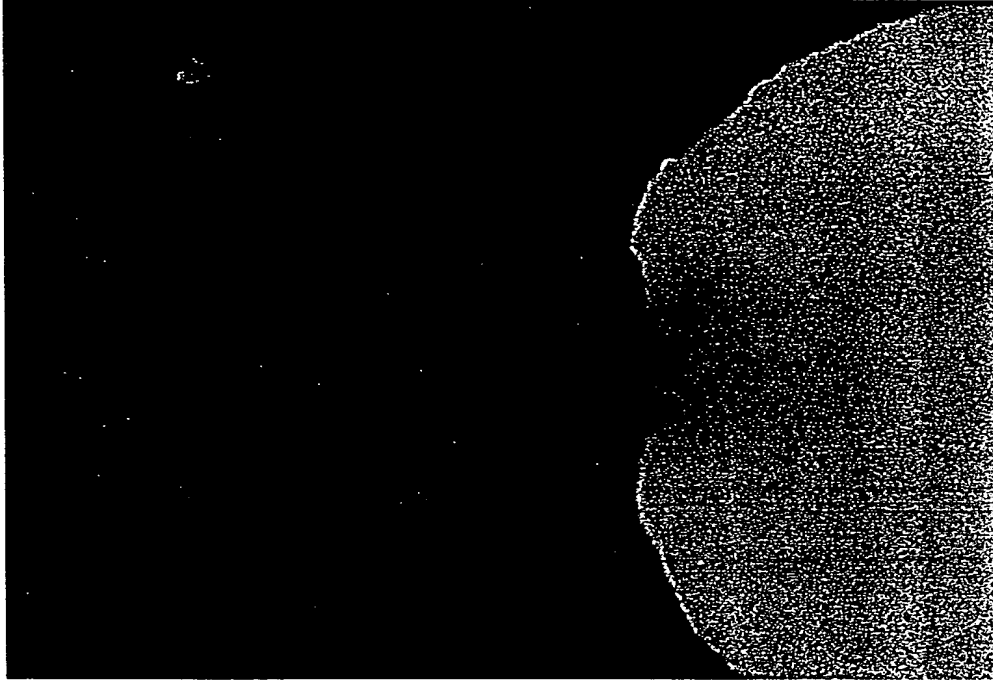


Figure 13

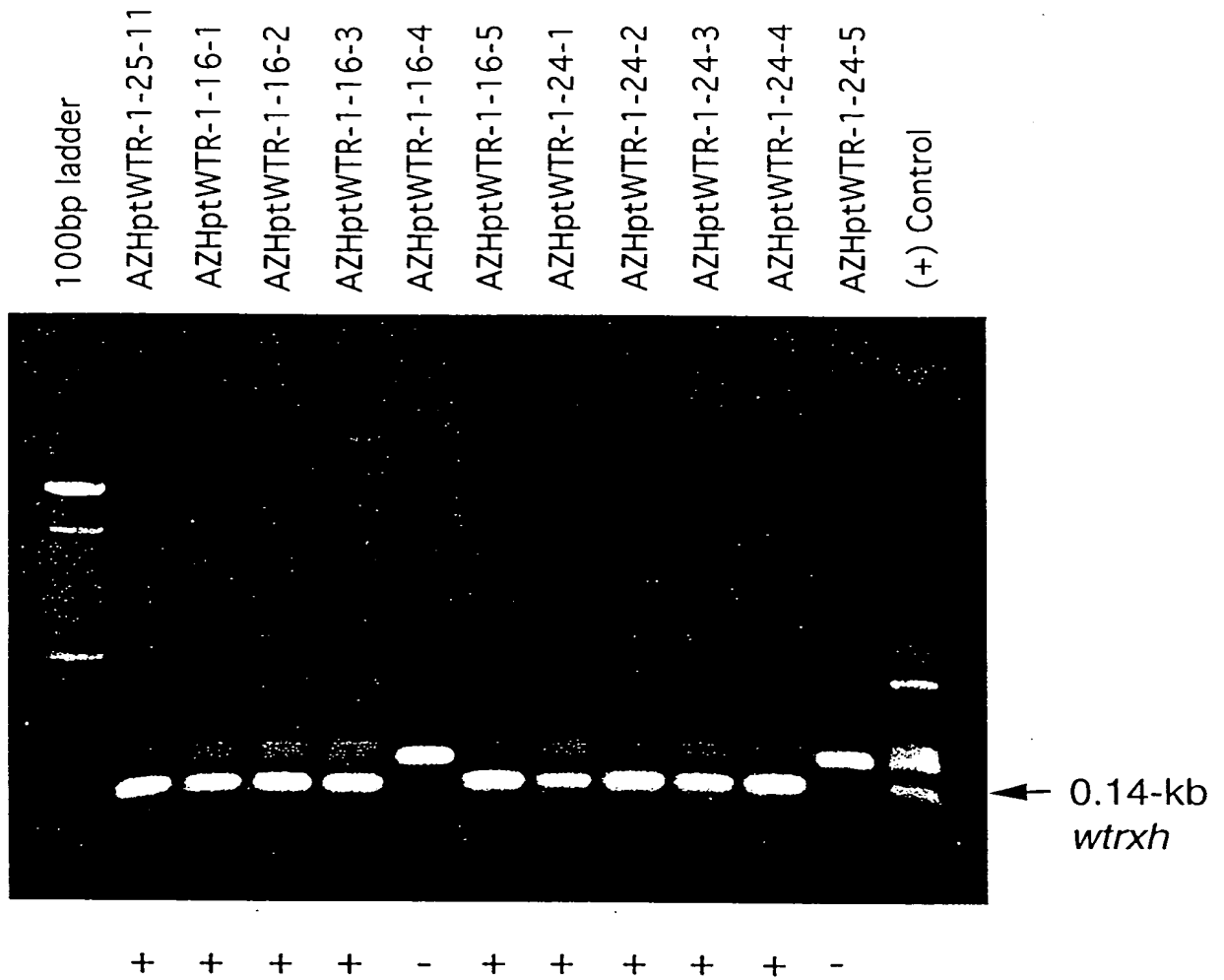


Figure 14

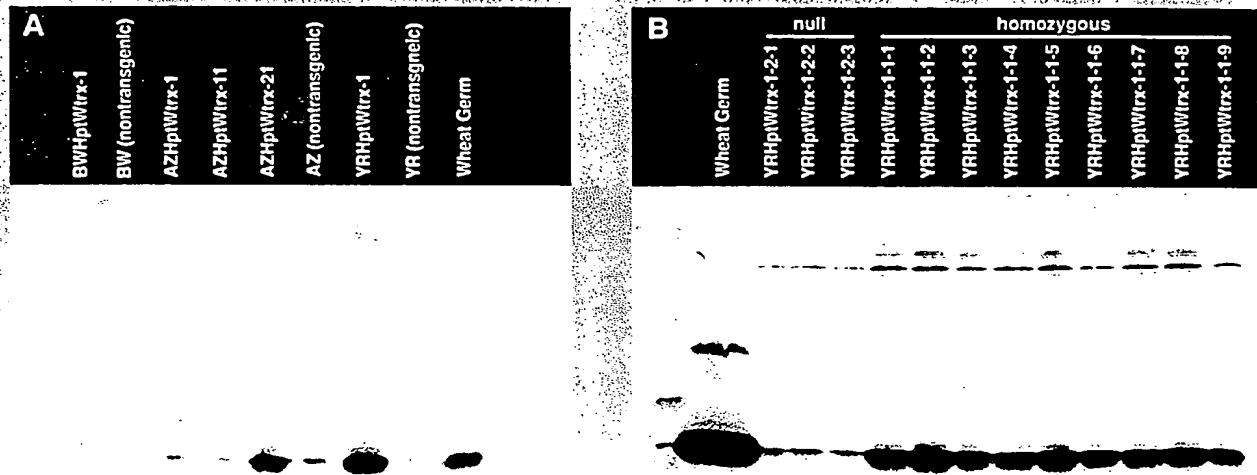


Figure 15

Figure 16

Effect of Thioredoxin Reduction on Digestion of
Wheat Glutenins by Trypsin

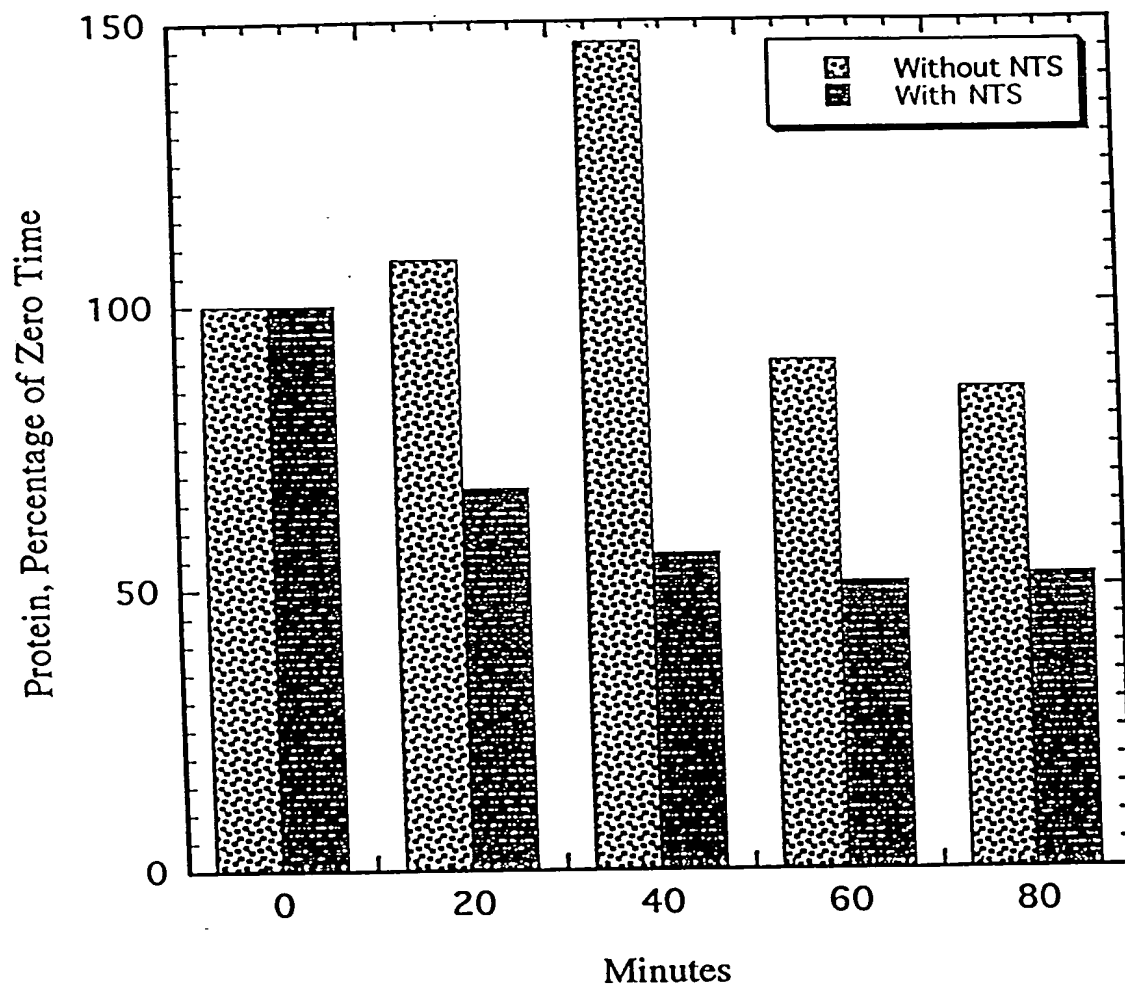
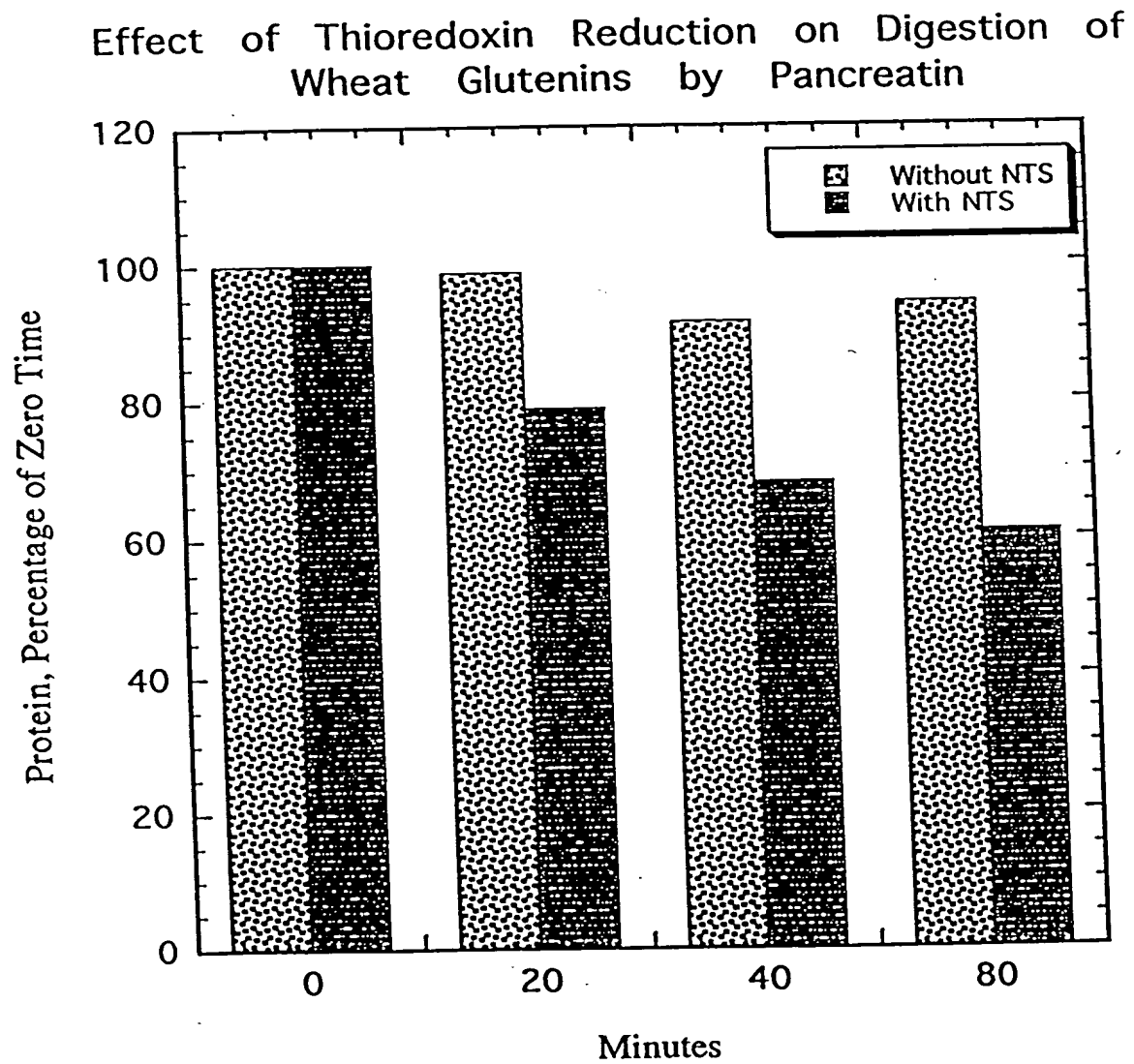
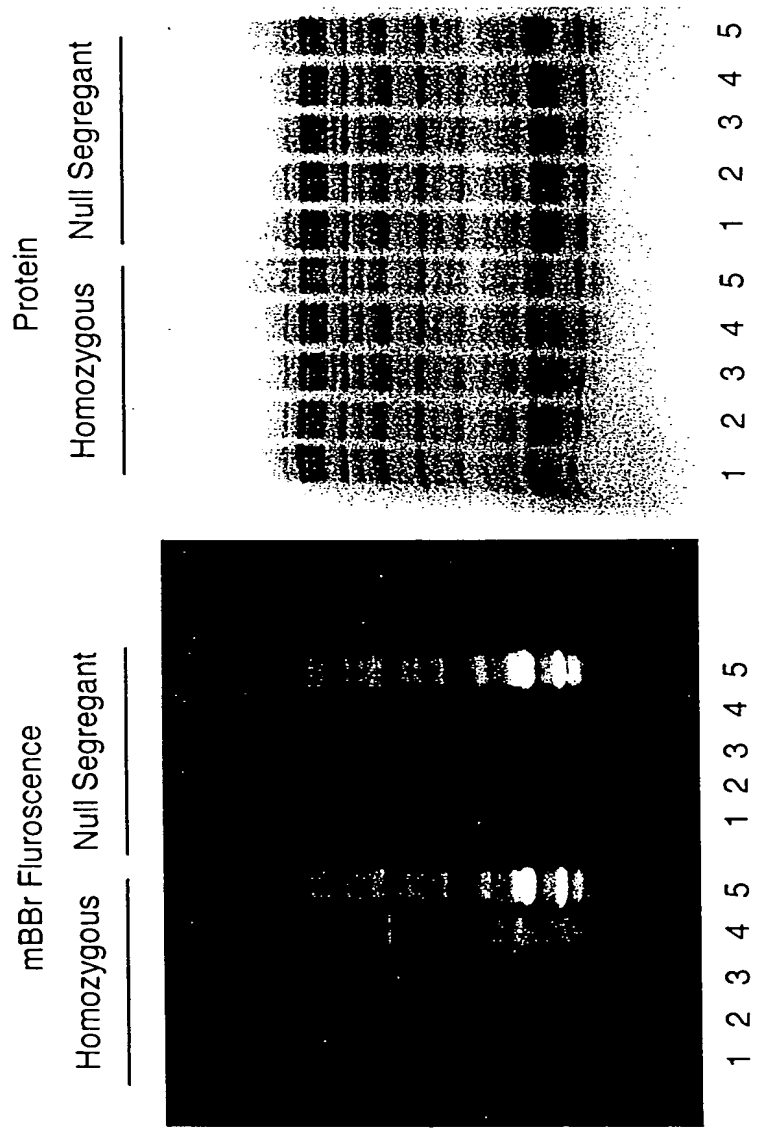


Figure 17



Effect of NTR on the Reduction of Proteins in Extracts of Wheat: Transgenic line Over-expressing Thioredoxin h vs. Null Segregant



- 1. Control
- 2. + NADPH
- 3. + NTR (*Arabidopsis*)
- 4. + NADPH & NTR
- 5. + DTT & boiled

Figure 18

Figure 19
Effect of Overexpressed Thioredoxin *h* on
Allergenicity of Proteins from Wheat Grain

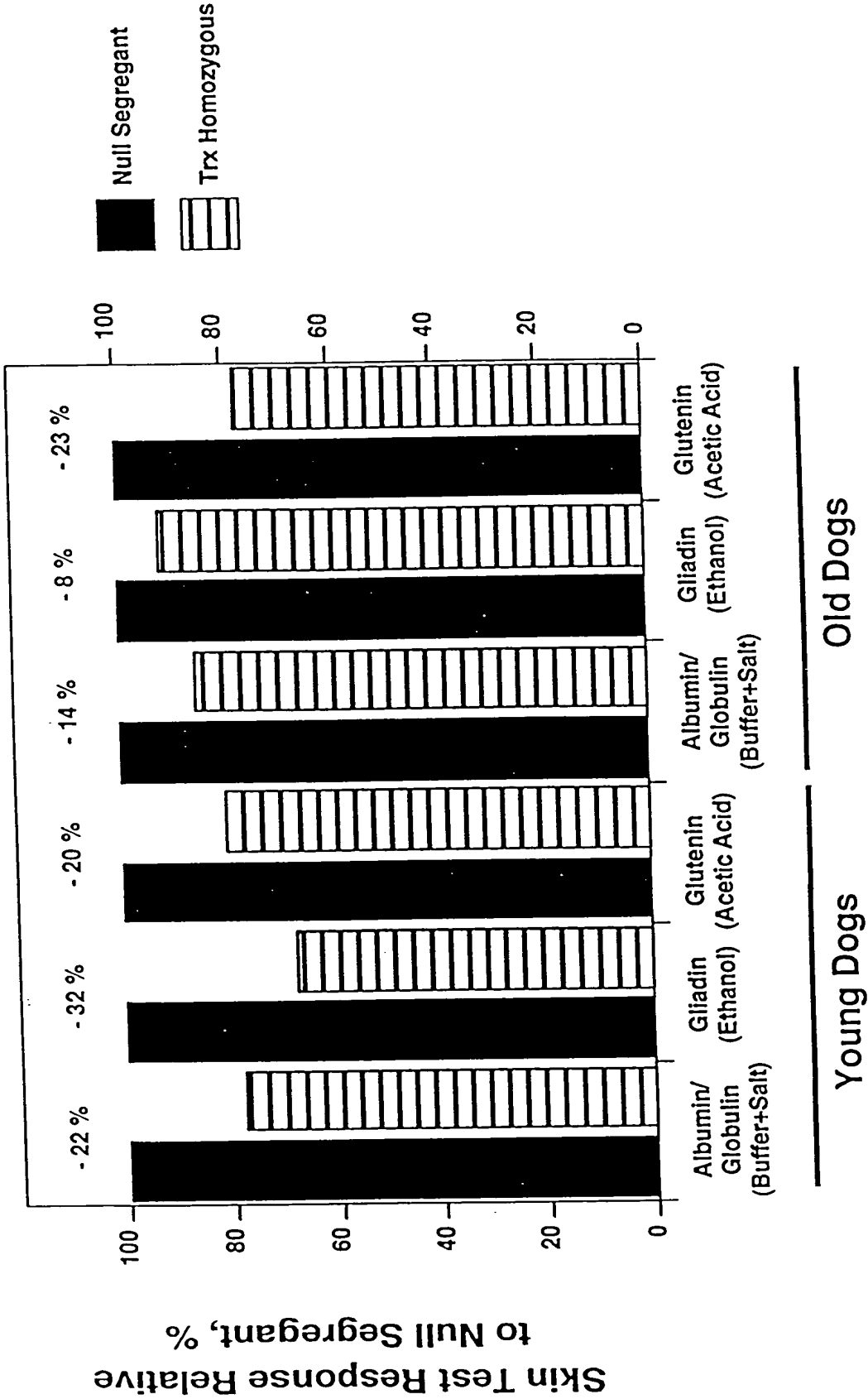


FIGURE 20

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atg gcg gcg tcg gca acg gcg gcg gca gtg gcg gcg gag gtg atc tcg	48
Met Ala Ala Ser Ala Thr Ala Ala Ala Val Ala Ala Glu Val Ile Ser	
1 5 10 15	
gtc cac agc ctg gag cag tgg acc atg cag atc gag gag gcc aac acc	96
Val His Ser Leu Glu Gln Trp Thr Met Gln Ile Glu Glu Ala Asn Thr	
20 25 30	
gcc aag aag ctg gtg gtg att gac ttc act gca tca tgg tgc gga cca	144
Ala Lys Lys Leu Val Val Ile Asp Phe Thr Ala Ser Trp Cys Gly Pro	
35 40 45	
tgc cgc atc atg gct cca gtt ttc gct gat ctc gcc aag aag ttc cca	192
Cys Arg Ile Met Ala Pro Val Phe Ala Asp Leu Ala Lys Lys Phe Pro	
50 55 60	
aat gct gtt ttc ctc aag gtc gac gtg gat gaa ctg aag ccc att gct	240
Asn Ala Val Phe Leu Lys Val Asp Val Asp Glu Leu Lys Pro Ile Ala	
65 70 75 80	
gag caa ttc agt gtc gag gcc atg cca acg ttc ctg ttc atg aag gaa	288
Glu Gln Phe Ser Val Glu Ala Met Pro Thr Phe Leu Phe Met Lys Glu	
85 90 95	
gga gac gtc aag gac agg gtt gtc gga gct atc aag gag gaa ctg acc	336
Gly Asp Val Lys Asp Arg Val Val Gly Ala Ile Lys Glu Glu Leu Thr	
100 105 110	
gcc aag gtt ggg ctt cac gcg gcg gcc cag taa	369
Ala Lys Val Gly Leu His Ala Ala Ala Gln	
115 120	

FIGURE 21

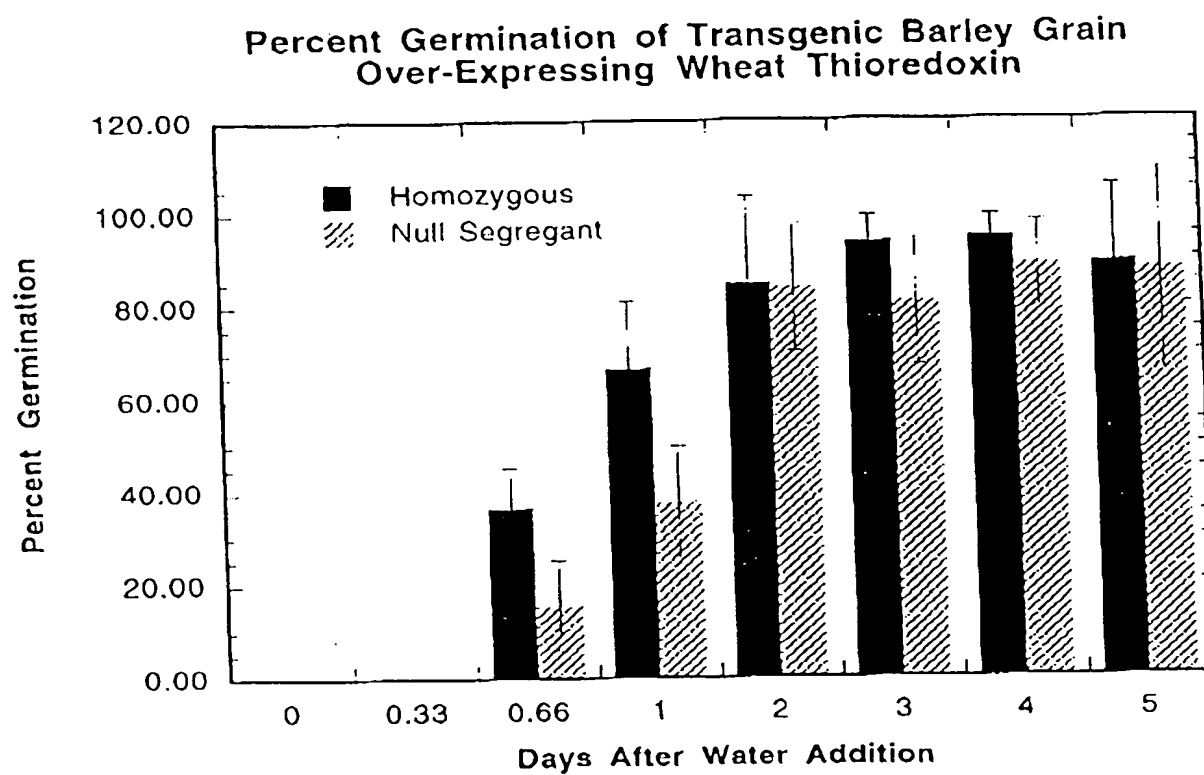
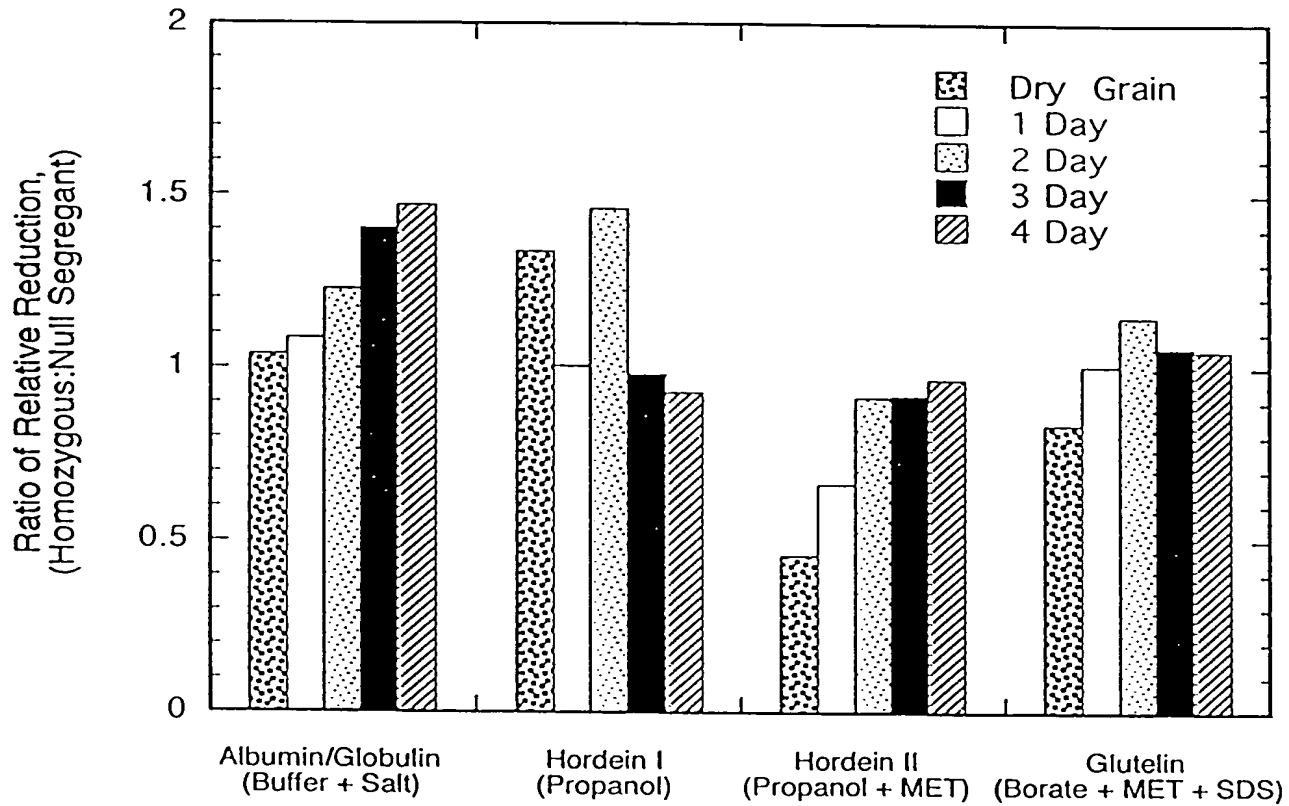


FIGURE 22

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**Different Relative Redox Status of Protein Fractions
in Transgenic Barley Grain Over-Expressing
Wheat Thioredoxin h vs. the Null Segregant:
Dry and Germinating Grain**



Effect of Glucose-6-phosphate Dehydrogenase on the Reduction of Proteins in Extracts of Transgenic Wheat Grain Overexpressing Thioredoxin *h* in the Presence of Glucose 6-phosphate and Arabidopsis NTR: +/- NTR

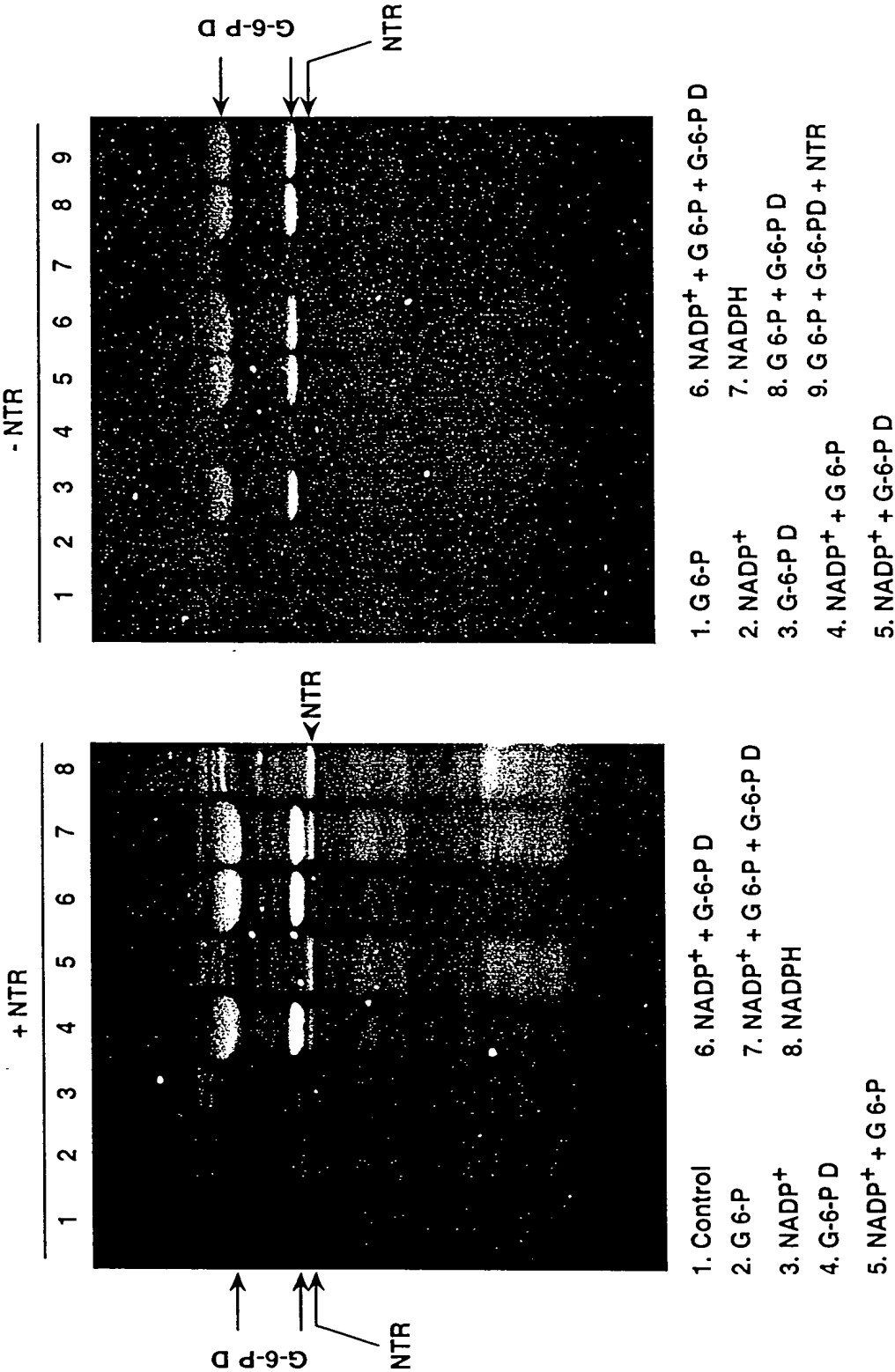


FIGURE 23

Effect of Glucose-6-phosphate Dehydrogenase on the Reduction of Proteins in Extracts of Null Segregant Derived from Wheat Grain Overexpressing Thioredoxin *h* in the Presence of Glucose 6- phosphate and Arabidopsis NTR: +/- NTR

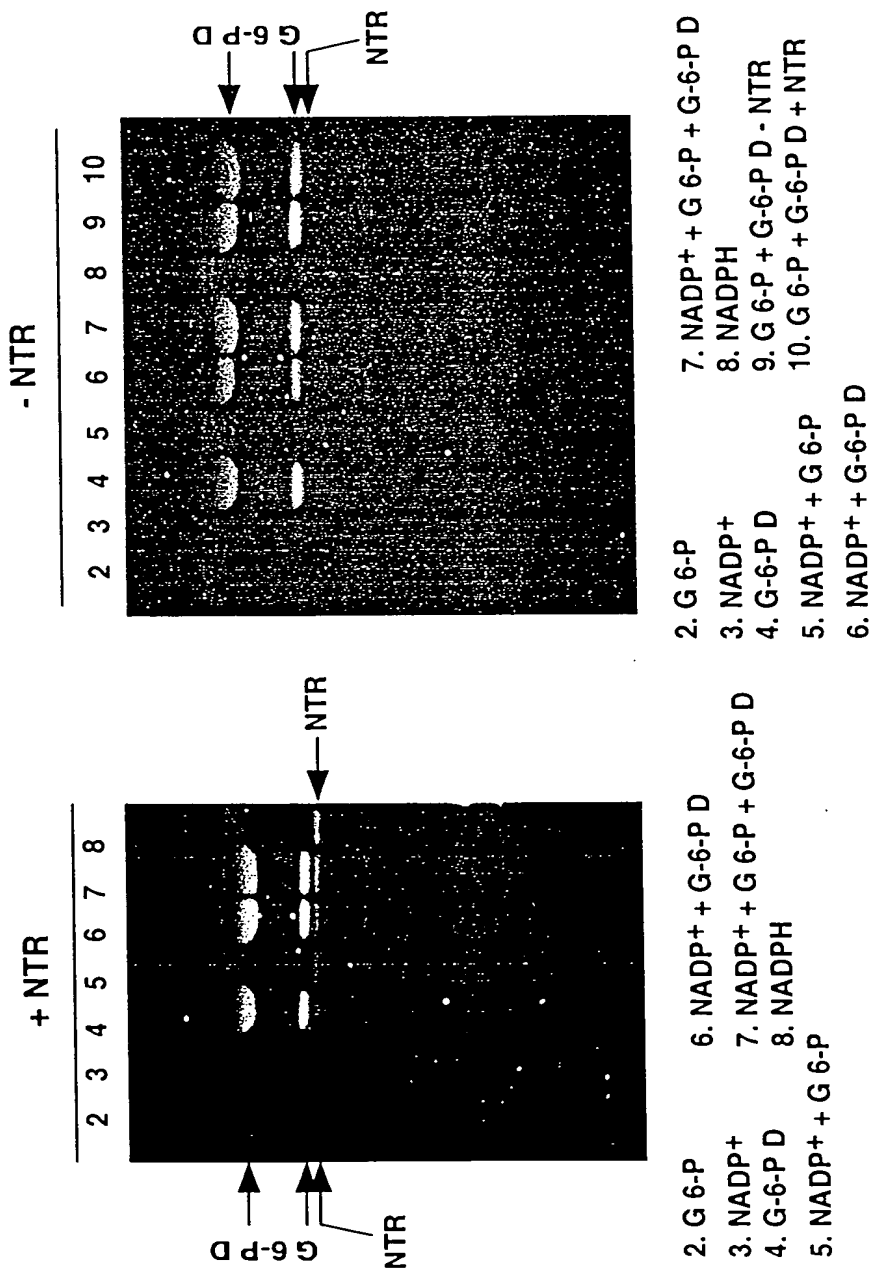


FIGURE 24